Influence of nuclear receptors on hepatocyte metabolism

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- Participation in the Virtual Liver Network:
  - A3.4: Linking signaling to metabolism
  - B5: Impact of inflammatory cytokines on ADME gene expression and drug detoxification
  - showcase steatosis

- Main focus on the development of quantitative models describing mutual influences across the levels of gene regulation, signaling, and metabolism

- Development of software and automated routines for simulation and model building

A3.4: Linking signaling and metabolism

- **Central question:**
  - Investigate the influence of selected endogenous and exogenous signaling pathways on intermediary metabolism and detoxification functions
  - Provide new solutions for the challenge of modeling the link between signaling cascades and metabolic networks
  - Develop models that cover the main links between signaling and metabolism with respect to qualitative and quantitative properties (e.g., input/output behavior)

- **Selected example:**
  - impact of nuclear receptor signaling on metabolism

- **Motivation:**
  - NRs have coordinating role for many physiological and pathophysiological functions of liver, especially metabolism
  - Research on NRs clearly underrepresented in VLN
  - NRs connect endogenous metabolism (e.g., lipids, energy) with drug detoxification

- **Specific goal:**
  - Analyze and model signaling effects of CAR, PPARα, and PXR on endogenous and drug metabolism in human hepatocytes by integrating transcriptomic, proteomic, metabolomic, enzyme activity, and genetic data
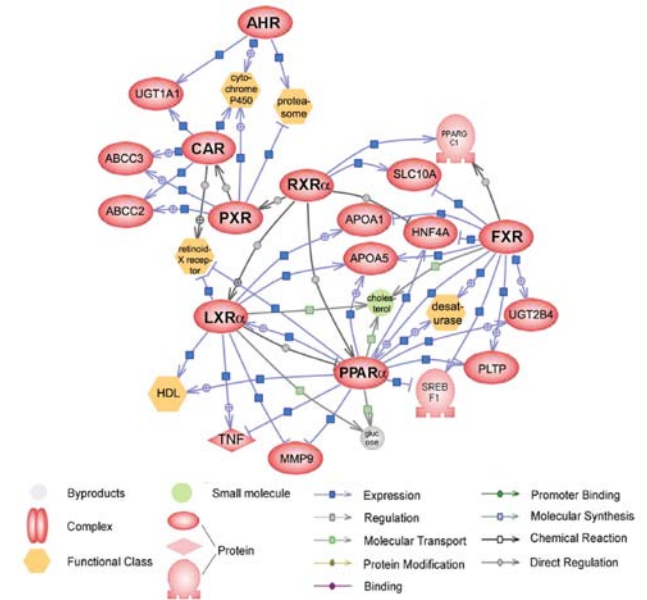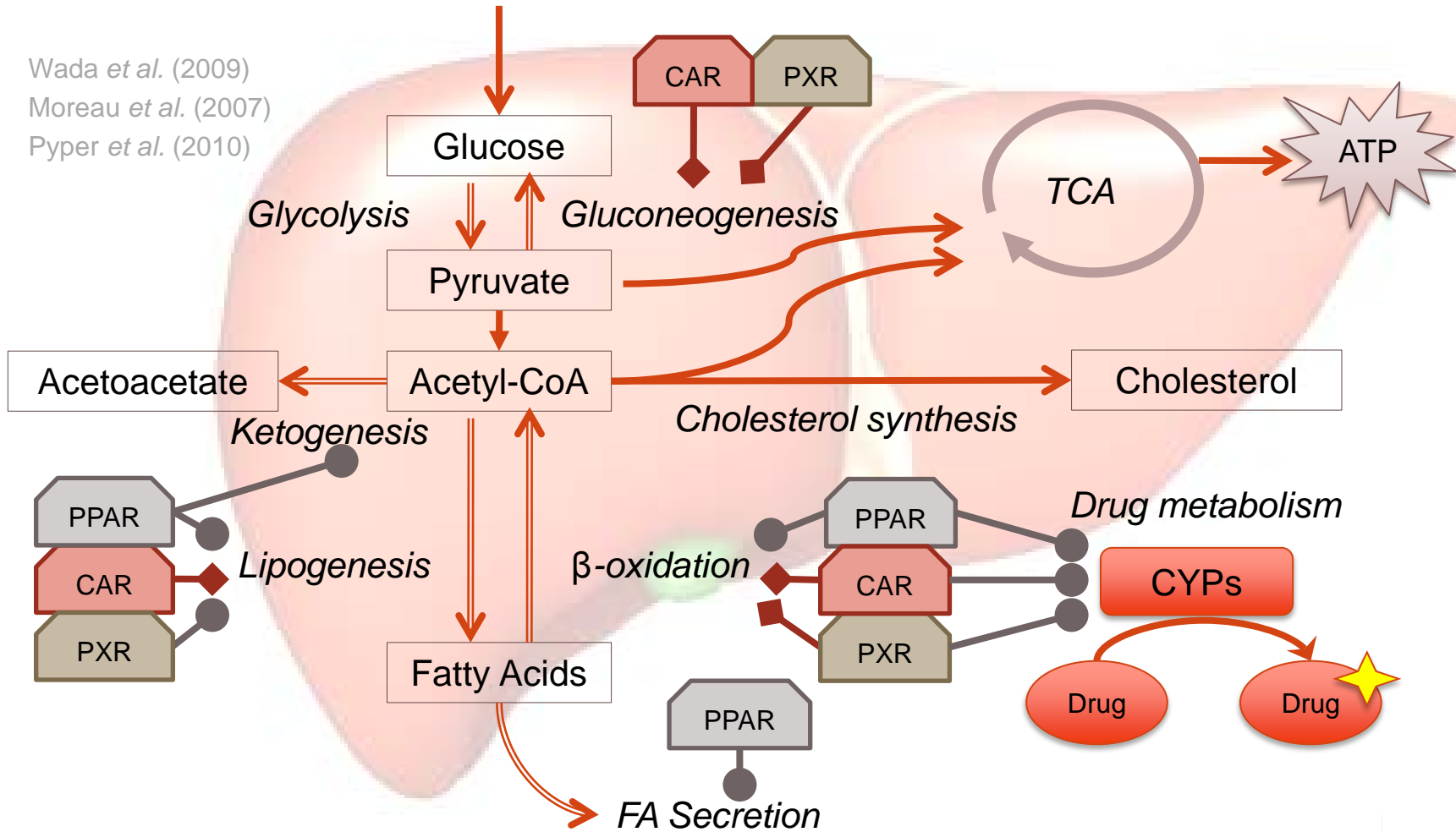

Cross-talk and co-regulation among nuclear receptors

Woods C G et al. Toxicol Pathol 2007;35:474-494

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Influence of CAR, PPARα and PXR on hepatocyte metabolism

Wada *et al.* (2009)
Moreau *et al.* (2007)
Pyper *et al.* (2010)

CAR   PXR

Glucose

Glycolysis   *Gluconeogenesis*

TCA

ATP

Pyruvate

Acetoacetate   Acetyl-CoA   Cholesterol

Ketogenesis   *Cholesterol synthesis*

PPAR

CAR   *Lipogenesis*   β-*oxidation*   PPAR   *Drug metabolism*

PXR   CAR   CYPs

PXR

Fatty Acids

PPAR   Drug   Drug

FA Secretion

Gene-regulatory effect of the nuclear receptors

Wagner *et al.* (2011): Nuclear Receptors in Liver Disease, Hepatology
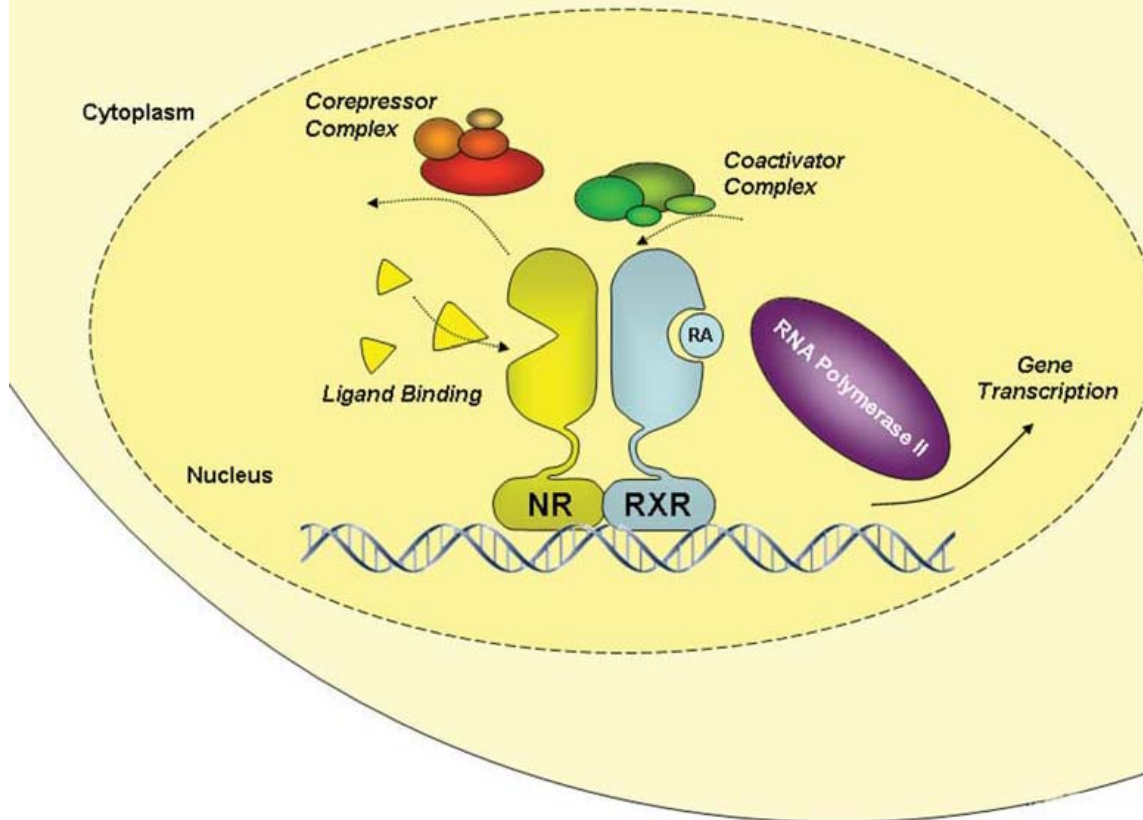
Legend	
NR	Nuclear Receptor
RA	Retinoic Acid
RXR	Retinoid X Receptor

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Experimental design and workflow

Agonists

Human Hepatocytes
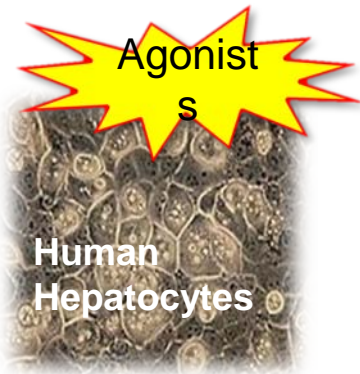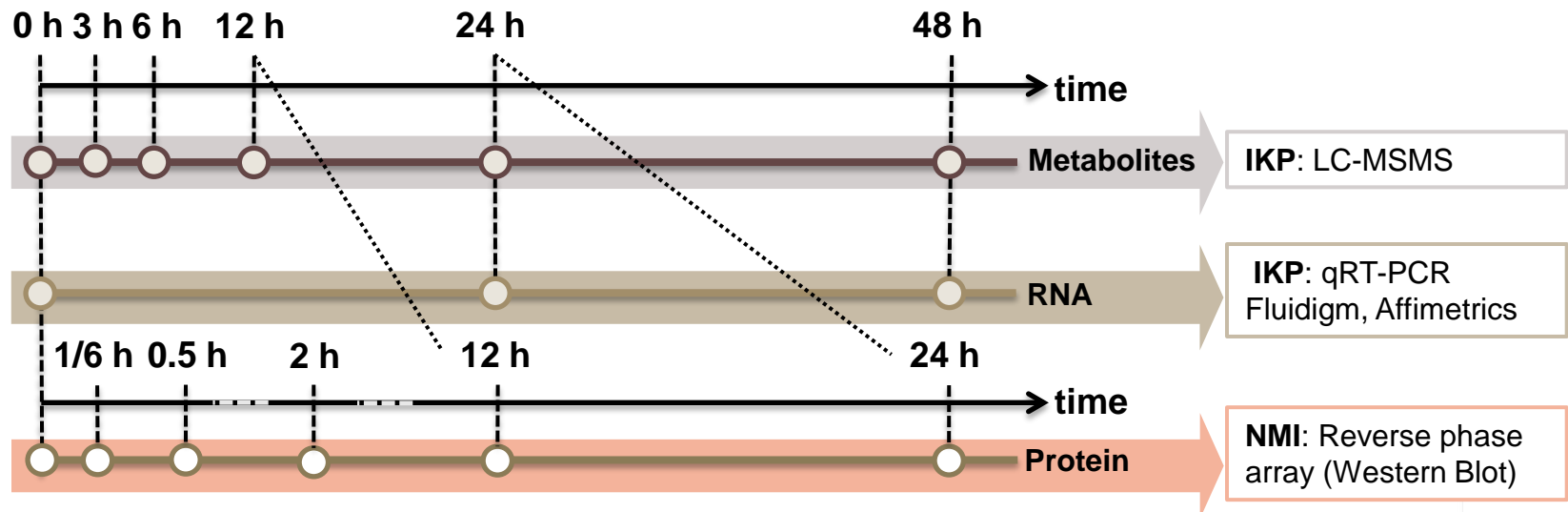
- NR-specific agonist/antagonist
- time-resolved readout of
  - high-quality quantitative transcriptomics (IKP)
  - (phospho-) proteomics of signaling pathway proteins (NMI)
  - metabolic profiles of lipids (triglycerides, phospholipids), bile acids, central metabolites, and drug metabolism activities (IKP)

Validation tools:
- knock-down with lentiviral-delivered shRNAs
- ChIP, microarray
- genotype-phenotype correlation

0 h	3 h	6 h	12 h		24 h			48 h	

time

○────○────○──────○──────────○──────────────────────○ **Metabolites**   **IKP**: LC-MSMS

○────────────────────────────○──────────────────────○ **RNA**   **IKP**: qRT-PCR Fluidigm, Affimetrics

1/6 h	0.5 h	2 h	12 h	24 h

time

○──○────○──────○──────────○──────────────────────○ **Protein**   **NMI**: Reverse phase array (Western Blot)

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Model of influence of nuclear receptors on metabolism

- Model explaining effects of nuclear receptor stimulation on metabolism

- Topology based on HepatoNet1 and KEGG database

- Current work: structural model refinement, flux analysis, and parameter estimation

- Extraction of known rate laws and parameters from SABIO-RK

- Automatic creation of missing Hill equations (gene regulation) and common modular rate laws using SBMLsqueezer

- In addition, gene-regulatory networks are under development for PXR, CAR, PPARα (currently without kinetic information) using Biobase data base (TRANSFAC), Ingenuity, literature

- Scheduled: Inclusion of protein phosphorylation states

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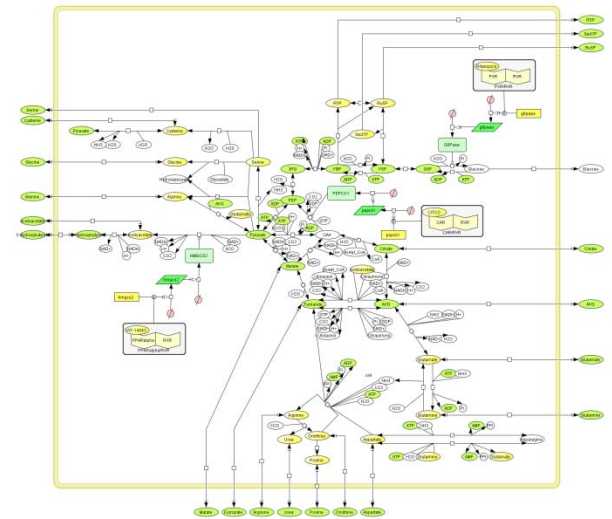
The influence of nuclear receptors on metabolism
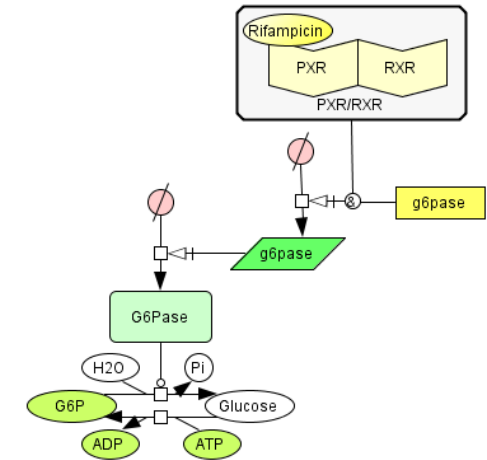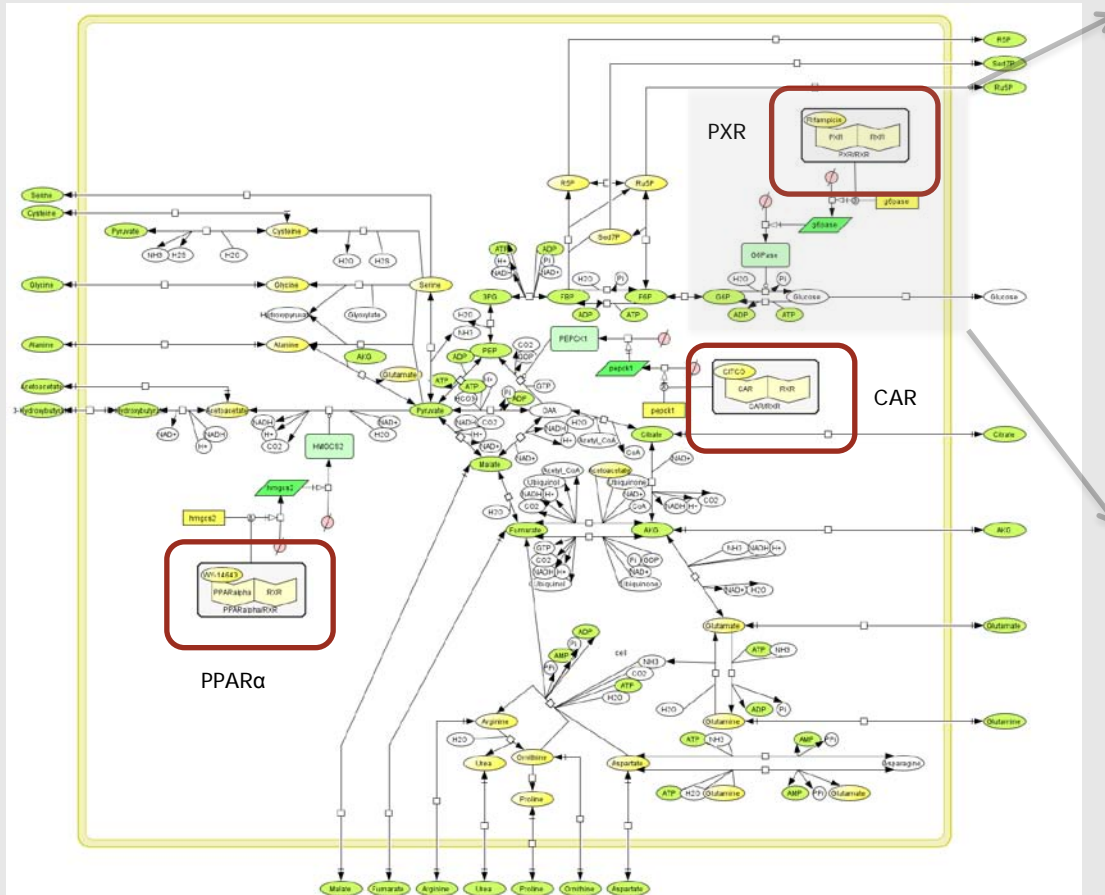
CAR, PXR, and PPARα act in transcription factor complexes influencing expression of enzymes in central liver metabolism

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Modeling software: KEGGtranslator

- Translating KEGG pathways to
  - GraphML
  - SBML
  - SBML with qual extension

- Improve KEGG annotations
  - Automated modelling
 - Easy linkage of analysis results to KEGG pathways
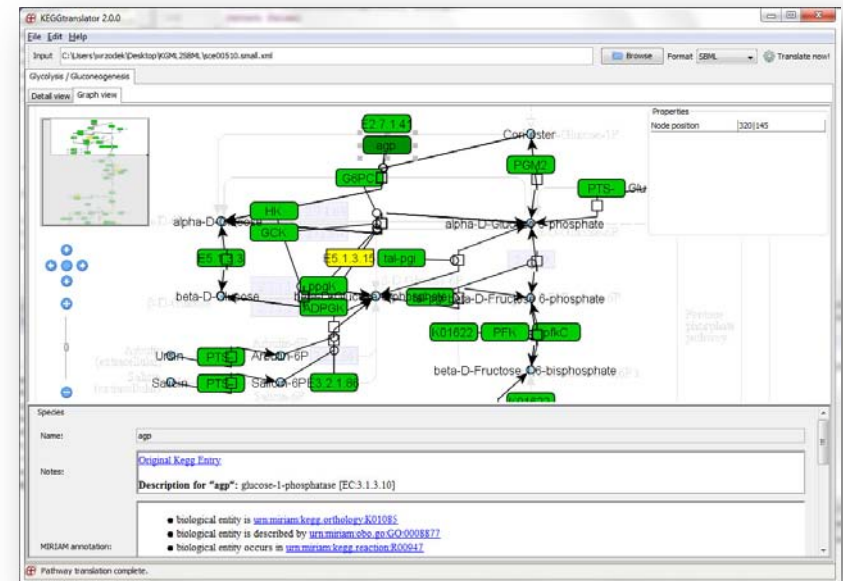
Wrzodek C, Dräger A, Zell A. KEGGtranslator: visualizing and converting the KEGG PATHWAY database to various formats, Bioinformatics, 2011.

http://www.cogsys.cs.uni-tuebingen.de/software/

Modeling software: kinetic law generator

- Development of software to complement structural models with kinetic equations
- SBMLsqueezer:
  - A powerful rate law generator for biochemical networks
  - Annotation function of SBML models using MIRIAM and SBO standards
- Application of SBMLsqueezer for the creation of a differential equation system from the structural models in this project
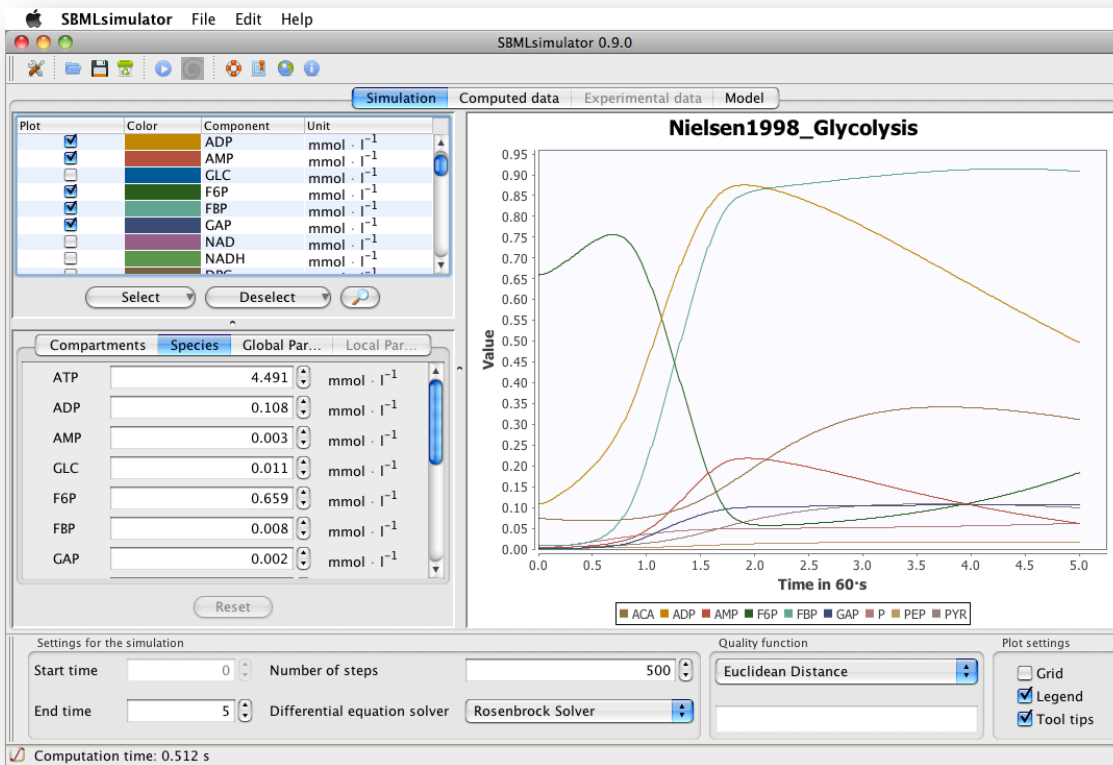- Internal data structure: JSBML
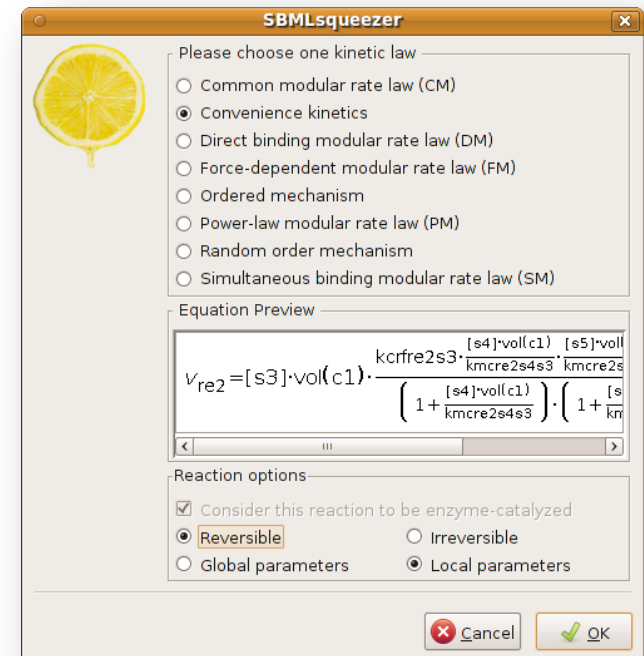
Dräger A, Hassis N, Supper J, Schröder S, and Zell A. SBMLsqueezer: a CellDesigner plug-in to generate kinetic rate equations for biochemical networks. *BMC Systems Biology*, 2(1):39, April 2008.

Modeling software: SBMLsimulator

Available for download at:
http://www.cogsys.cs.uni-tuebingen.de/software/SBMLsimulator/

Some key features

- **Accurate**: 100 % of the SBML Test Suite (980 models)
- **Fast**: 500 integration steps of the glycolysis (Biomodel 42) computed in 0.512 s.
- **JSBML-based** (Java library for SBML)
- **Platform independent**: Tested on Windows, Linux, and Mac OS
- **Bilingual user interface** (English and German)
- All program features also available through the **command-line interface**
- **Nine** numerical **integration methods**, including the Rosenbrock Solver for stiff systems
- Heuristic **optimization framework EvA2** included

Modeling software: SBMLsimulator

Available for download at:
http://www.cogsys.cs.uni-tuebingen.de/software/SBMLsimulator/
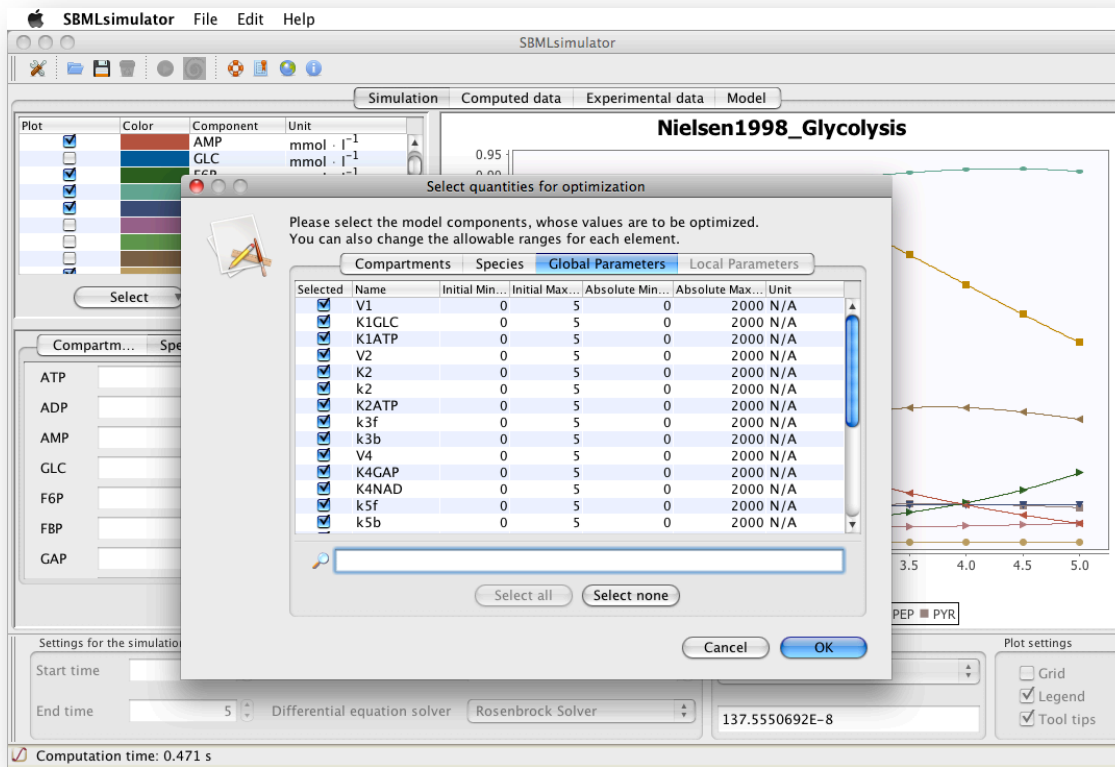
- Loading one or multiple experimental data sets
- Selection of optimization targets and quality measure
- Search function for easier selection of model components
- Multiple shooting and single shooting integration strategy possible
- Selection of the desired optimization procedure

Modeling software: SBMLsimulator with EvA2

Available for download at:
http://www.cogsys.cs.uni-tuebingen.de/software/SBMLsimulator/
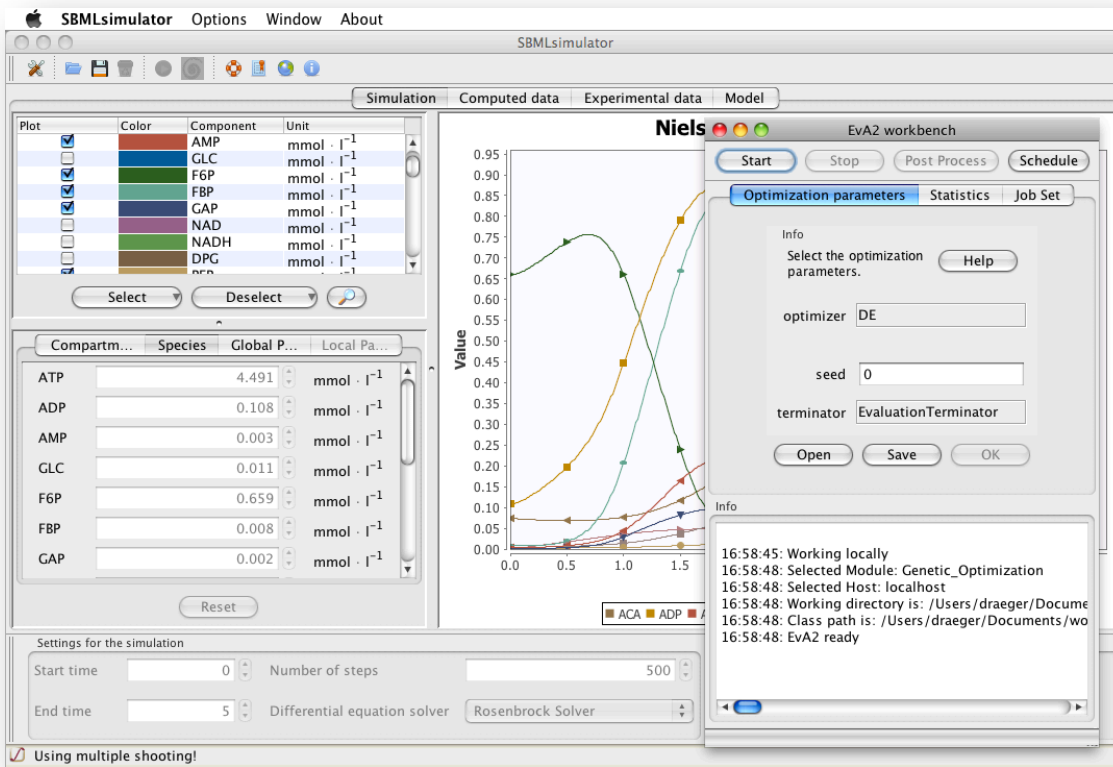

- Nature inspired heuristic optimization algorithms included:
  - Genetic Algorithm
  - Simulated Annealing
  - Particle Swarm Optimization
  - Differential Evolution
  - Evolution Strategy
  - Evolution Strategy with Covariance Matrix Adaptation
  - Monte Carlo Optimization
  - Tribes
  - Multistart Hill Climber
- Each algorithm with a large number of settings
- Dynamic update between optimization results and graphical user interface
- Eight quality measures included, e.g., Euclidean Distance and Relative Squared Error

Challenges

- Large number of parameters with uncertain values and the question of identifiability

- Different time scales (gene expression and metabolism)

- Extension of SBML for qualitative relationships (qual package)

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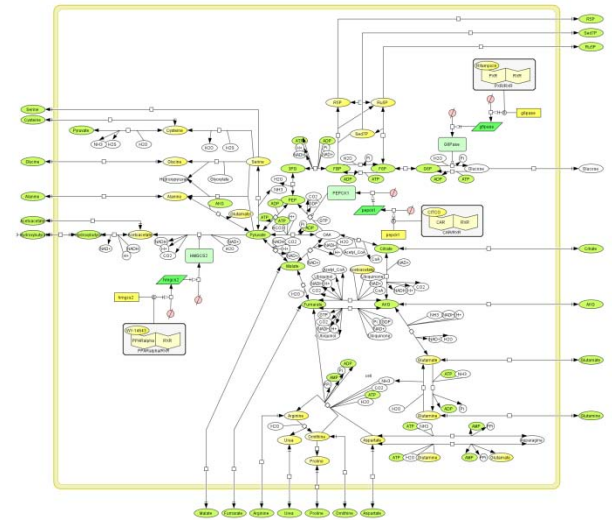
Publications

(1) Schröder A. *Inference of gene-regulatory networks in primary human hepatocytes*. PhD thesis, University of Tuebingen, Tübingen, Germany, November 2011.

(2) Schröder A, Klein K, Winter S, Schwab M, Bonin M, Zell A, and Zanger UM. Genomics of ADME gene expression: mapping expression quantitative trait loci relevant for absorption, distribution, metabolism and excretion of drugs in human liver. *The Pharmacogenomics Journal,* pages 1473-1150, September 2011.

(3) Schröder A, Wollnik J, Wrzodek C, Dräger A, Bonin M, Burk O, Thomas M, Thasler WE, Zanger UM, and Zell A. Inferring statin-induced gene regulatory relationships in primary human hepatocytes. *Bioinformatics*, 27(18):2473-2477, July 2011.

(4) Dräger A, Rodriguez N, Dumoussea M, Dörr A, Wrzodek C, Le Novère N, Zell A, and Hucka M. JSBML: a flexible Java library for working with SBML. *Bioinformatics*, 27(15):2167-2168, June 2011.

(5) Schröder A, Wrzodek C, Wollnik J, Dräger A, Wanke D, Berendzen KW, and Zell A. Inferring transcriptional regulators for sets of co-expressed genes by multi-objective evolutionary optimization. In *IEEE Congress on Evolutionary Computation (CEC 2011)*, New Orleans, USA, June 2011.

(6) Dräger A. *Computational Modeling of Biochemical Networks*. PhD thesis, University of Tuebingen, Tübingen, Germany, January 2011.

Thank you for your attention!

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